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Comparative Biology of Test Species

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This paper assesses the capacity of animal models to predict human response to carcinogenic agents with consideration for the heterogeneity of humans. It is widely accepted that human susceptibility to toxic substances, including carcinogens, is highly variable. Conventional rodent models are usually highly inbred and valued for their ability to display characteristic homogeneity. Current practice assumes that the homogeneity of response to toxic agents, including carcinogens, in the rodent model will be representative of humans. The issue then becomes, To which of the broad spectrum of human responses are specific animal models likely to be related?

This paper examines the extent of human heterogeneity over a broad range of biochemical characteristics (e.g., aryl hydrocarbon hydroxylase activity, epoxide hydrase activity, β-glucuronidase activity, debrisoquine hydroxylation, DNA-adduct formation) with emphasis on those biochemical characteristics that affect responses to carcinogens. Examples are presented to compare the heterogeneity of selected animal models for these biochemical characteristics as they relate to the spectrum of human responses noted above. The paper presents a theoretical perspective for determining to which part of the human population response spectrum common animal models are most likely to be extrapolated.

Introduction

There are a number of biological factors that may enhance an individual's susceptibility for adverse health effects from exposure to toxic substances, including carcinogens. These factors include age, sex, genetic composition, nutritional status, and preexisting disease conditions (*1–5*). The magnitude to which predisposing factors enhance susceptibility to toxic substances is known only to a limited extent. The conventional wisdom employed in regulatory toxicology has been to assume that most interindividual variations in response to noncarcinogenic toxic substances can be accounted for by a factor of 10 (*6*). This position was supported in part by Dourson and Stara (*7*), who reviewed the acute toxicity response of rodents to a number of toxic agents. For 92% of the compounds studied, they found that a 10-fold decrease in dose at the LD_{50} level would reduce the exposure below the general range expected to result in death. Inasmuch as these ranges were obtained with inbred strains of laboratory rats, it is expected that these rodents would have less variation in response to toxic substances than the highly outbred and more heterogeneous human population.

The heterogeneity of human response to toxic agents would be expected to be much greater than that in the widely used and highly inbred rodent model strains. An important factor that needs to be documented is the relationship between the limited capacity of highly inbred strains of rodents to predict accurately the range of human response and the extent of interindividual variation among humans in response to toxic agents. This information is necessary to establish the biological basis of the uncertainty (safety) factor of 10 currently recommended for within human species variation by the National Academy of Sciences (*6*) and used by the U.S. Environmental Protection Agency (*8*) in establishing acceptable exposure standards for noncarcinogens. Unfortunately, the recommended use of such an uncertainty factor by the National Research Council (*6*) has not been accompanied by supportive documentation. The numerous problems related to the heterogeneity of human responses to carcinogenic agents becomes apparent with the understanding that current procedures for quantitative risk assessment apply biomathematical models to bioassay data. This procedure assumes that the human distribution of sensitivities will be described by the response of inbred animal models. Current approaches for predicting human cancer risks from animal studies do not consider human heterogeneity.

Variation in Response to Selected Xenobiotics

The following examples will examine the range of human responses with respect to the degree of variation in xenobiotic metabolism and other relevant end points associated with susceptibility to carcinogenic agents. The data presented will establish the range of human variation in a number of parameters thought to affect susceptibility to cancer-causing agents. A similar comparison of animal variation will be made and then compared to the human response. This comparison permits an assessment of relationships between the range of

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human responses to the responses of various animal models. On the basis of this information the paper addresses the question, To what part of the spectrum of human population variation do specific animal models extrapolate?

Acetylation

Human. Certain aromatic amines are known to be both animal and human carcinogens. These substances tend to be metabolized by acetylation to compounds that are less potent carcinogens. Both fast and slow acetylators have been identified within the human population. In the United States, the distribution is roughly equal (i.e., 50%/50%) among both Caucasians and blacks (*2*). An assessment of the acetylation of about a dozen aromatic amines by humans revealed a variation in response of from 3.7- to 13.0-fold (*9*).

Animal. Lower and Bryan (*10*) assessed the relative capability of *N*-acetyltransferase enzyme systems from liver cytosols of various mammalian species (i.e., hamsters, guinea pigs, mice, and rats) to transfer acetyl groups from acetyl-CoA to the amine function of the carcinogens 2-aminofluorene (2-AF), 4-aminobiphenyl, and 2-aminonaphthalene. The most readily acetylated of these carcinogens in all four species was 2-AF, followed by 4-aminobiphenyl and 2-aminonaphthalene. The highest enzyme activity was present in the liver cytosol of the hamster, followed in order by the guinea pig, mouse, and rat. Relative to the rat, which is arbitrarily given 1 as a value, the mouse is 8, the guinea pig is 12, and the hamster is 18. In contrast, dog liver cytosol was incapable of carrying out detectable *N*-acetylation of any of the three carcinogenic arylamines studied.

Among the four commonly used animal models, the capacity for the hamster to acetylate 2-AF is 9- to 10-fold higher than a human fast acetylator and 120-fold greater than a slow human acetylator. Guinea pigs and mice were 5.7- and 4.6-fold more efficient acetylators than the fast human acetylator for AAF. In marked contrast, the rat displayed a capacity to acetylate 2-AF in a manner that was intermediate to both the human fast and slow acetylator phenotypes. More specifically, the rat displayed about a 7-fold greater activity than the human slow acetylator and about 53% of the activity of the fast acetylator.

In contrast to the species discussed, it is well known that rabbits and humans display *N*-acetyltransferase (NAT) activity for isoniazid (INH) and several other arylamine drugs, including sulfapyridine, hydralazine, and procainamide. The activity of this enzyme is subject to a genetic polymorphism that can be expressed as fast or slow acetylators. Genetic differences in the rate of *N*-acetylation are associated with individual susceptibility to toxicity from the chronic administration of drugs like INH. In the case of INH, slow acetylator humans are known to be at increased risk for developing peripheral neuropathies (*11*); fast acetylators have an enhanced risk of developing hepatitis (*12*). Lower et al. (*13*) hypothesized that the acetylator phenotype is a risk factor in the development of arylamine-induced bladder cancer in humans. Evidence from these studies indicates that the *N*-hydroxylated nonacetylated metabolite of the arylamine is the bladder carcinogen. The risk for bladder cancer may be a function of the individual's capacity to acetylate and deacetylate arylamines following *N*-hydroxylation.

The recognition that both rabbits and humans display a genetic polymorphism with respect to acetylation has led to speculation that the rabbit may be a predictive animal model for population-based acetylation responses. Glowinski et al. (*9*) compared the rates of acetylation in both fast and slow acetylator phenotypes in rabbits and humans for seven agents, including five arylamine carcinogens: α-naphthylamine, benzidine, β-naphthylamine, 2-AF, and methylenebis(*O*-chloroaniline) (MOCA). In general, it was found that the fast acetylator rabbit displayed a rate of activity of from 10- to 50-fold greater than the fast acetylator human. The acetylation for the slow acetylator rabbit ranged from 1/90 to 1/580 of the fast rabbit acetylator. However, the rabbits' acetylation activity levels were generally in the range displayed by both the fast and slow human acetylator, depending on the substrate. For example, the slow acetylator rabbit was very similar to the slow human acetylator for 2-AF (0.013 vs. 0.021 μmole/mg/hr) and benzidine (0.016 vs. 0.019 μmole/mg/hr), but more closely comparable to the human fast acetylator for β-naphthylamine (0.28 vs. 0.23 μmole/mg/hr).

The net result of such a comparison is that the rat [Sprague-Dawley (SD) female] and the slow acetylator New Zealand rabbit (both sexes) displayed *N*-acetylation activity for several substrates thought to be carcinogens in the range of both human fast and slow acetylators, depending on the substrate. In general, the hamster, guinea pig, and mouse displayed acetylation activity that was markedly greater than that displayed by the human fast acetylator. The one exception to this is seen for the arylamine α-naphthylamine, where the extent to which these three species exceeded the fast human acetylator phenotype was not greater than 2.5-fold.

Deacetylation

Animal. The capacity to deacetylate carcinogenic arylacetamides has been shown by Lower and Bryan (*10*) to be a significant risk factor affecting the occurrence of bladder cancer in the dog. This hypothesis is derived from observations indicating that the *N*-hydroxy-nonacetylated metabolite is the carcinogenic agent. The dog displays an increased risk of bladder cancer because this species has the ability to *N*-hydroxylate the aromatic amines but has a limited capacity to acetylate such compounds. Similarly, with exposure to carcinogenic arylacetamides, the dog has an enhanced risk of bladder cancer from the capacity to *N*-hydroxylate the agent and then to deacetylate it to the carcin-

ogen. It is important to recognize that while nonacetylated arylamines that have been *N*-hydroxylated are likely candidates for bladder carcinogens, the *N*-hydroxylated acetylated arylamines are likely candidates for liver carcinogens. These examples show that enzymes carrying out the *N*-acetylation of arylamines and deacetylation of arylacetamides play an important role as determinants of susceptibility to liver and bladder cancer.

The relative capacity of the animal model to acetylate and deacetylate is then an important variable with respect to arylamine-induced cancer. In the rat and mouse, the ratio of *N*-acetyltransferase to deacetylase activity is very high relative to the dog. This may provide rodents with some protection against bladder cancer, but not for hepatocarcinogenesis (*10,14*).

Human. Little is known about the range of variation with respect to deacetylation in humans. However, *in vivo* studies with acetanilide indicate that humans and rabbits, in contrast to dogs, are poor deacetylators of acetylated aromatic amines (*15*).

Given the greater ability of humans to acetylate than the dog and the lower human capacity to deacetylate aromatic amines, it is likely that humans, especially the fast acetylator phenotypes, would be at considerably lower risk of developing an arylamine bladder cancer than dogs.

Debrisoquine Oxidation

Human. Among humans, interindividual variations exist with respect to the oxidation of carbon centers in several drugs, including debrisoquine, guanoxan, and phenacetin. With respect to debrisoquine, Idle and Smith (*16*) have reported that this guanidine-based antihypertensive drug is metabolized by humans principally via alicylic oxidation to yield 4-hydroxydebrisoquine (Fig. 1). The metabolic process is under the control of a single autosomal gene (*17,18*). Mbanefo et al. (*19*) suggest that at least two alleles occur in the population that can affect the extent of this reaction. The two alleles have been designated D^H (extensive hydroxylation of debrisoquine) and D^L (low hydroxylation). Homozygote individuals for the D^L allele show a significantly reduced capacity to 4-hydroxylate debrisoquine and excrete the substance, for the most part, unchanged. Individuals homozygous for the D^L allele make up the poor-metabolizing (PM) phenotype (*19*). Individuals who are designated as genotypically homozygous or heterozygous for D^H are known to readily hydroxylate debrisoquine. These are both extensive-metabolizing (EM) phenotypes. The quotient, percent dose eliminated as unchanged debrisoquine/percent dose excreted as 4-hydroxydebrisoquine in the urine after a single 10-mg oral dose, is bimodally distributed and referred to as the "metabolic ratio." The EM phenotype exhibits metabolic ratios in the range of 0.01 to 10.0; the PM phenotype displays metabolic ratios in the range of 18 to 200 (Fig. 2). If one considered the extreme range of values given for the metabolic ratios of 0.01 to 200, a difference of 20,000-fold metabolic variation is seen in humans. However, it should be pointed out that the majority (75–85%) of persons tested fall within a 10-fold range (*16*).

Animal. With respect to animal models, Al-Dabbagh et al. (*20*) assessed the extent of animal modeling of human polymorphic drug oxidation using the metabolism of debrisoquine and phenacetin in rat strains. Marked interstrain differences with respect to the metabolic ratio were determined. The DA-strain rat displayed a metabolic ratio for debrisoquine of 4.7, whereas the Lewis rat had a metabolic ratio of 0.29 (Fig. 3).

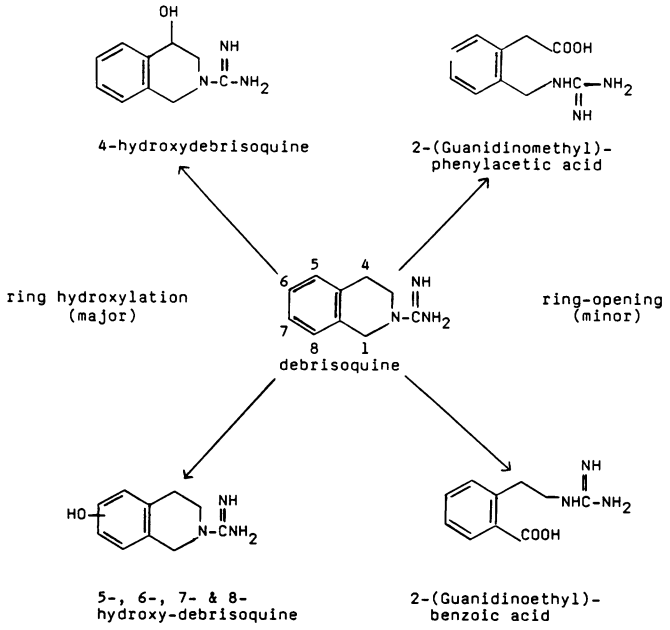

FIGURE 1. Metabolic disposition of debrisoquine in man. Modified from Idle and Smith (*16*).

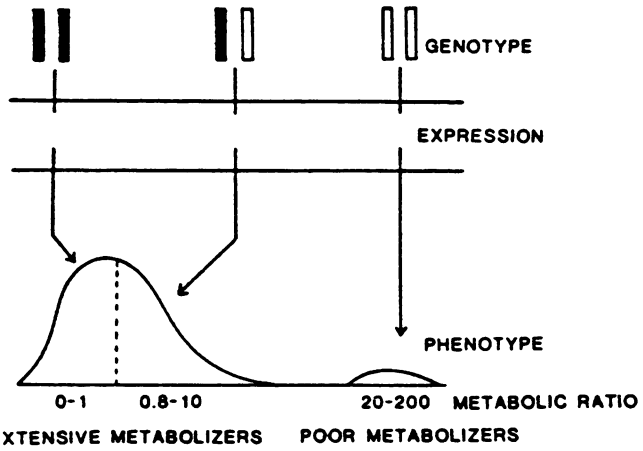

FIGURE 2. The genetic model in which three genotypically distinct allele pairs give rise to two distinct phenotypes (*16*).

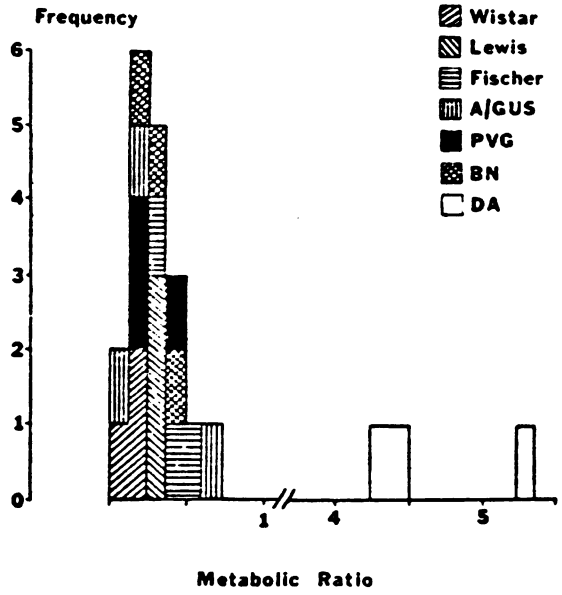

FIGURE 3. Frequency distribution histogram of metabolic ratios (% debrisoquine/% 4-hydroxydebrisoquine) for seven rat strains (*20*).

These values tended to simulate the activity for human PM and EM, respectively.

Aryl Hydrocarbon Hydroxylase (AHH) Variation

Human. Kouri et al. (*21*) reported about a 40-fold interindividual variation of benzo[a]anthracene-induced AHH activity in mitogen-activated human lymphocytes (n = 300 people). In addition, Harris et al. (*22*) reported that interindividual differences in AHH activities also occur in other tissues, including the placenta (several hundredfold) and the skin, kidney, and bronchus (less than 20-fold). With respect to hepatic variation of AHH, Pelkonen et al. (*23*) reported a variation of 16-fold based on samples from patients with various types of disorders (e.g., cholelithiasis, icterus, cancer, liver injury).

Animal. Pelkonen et al. (*23*) assessed the level of AHH activity in livers from adult humans, different strains of rats (noninbred SD, inbred SD, DA, Fischer, and Wistar), guinea pigs, and rabbits. Based on mean values, the activity of AHH in human liver was about 30 to 60% that of the male rat liver, depending on the strain of rat. No sex differences occur for humans, but the female rat displays about 20 to 40% of the hepatic activity of the male rat.

The range of human values was from approximately 20 to 320 pmole/g liver/min, with the mean being 100 units. This places the low human values in the range of the female inbred rat, which has activity from about 15 to 20. The upper range of human activity is in the activity zone of the male rat. Therefore, the upper and lower tails of the human distribution curve appear to be in the range of activity of the animal model.

Examination of rabbit data revealed a generally similar but somewhat lower (~60 units) activity than in humans, along with no sex difference. It is interesting to note that rabbits, like humans, are highly outbred and that species outbreeding may have contributed to the nearly 6-fold range in rabbit AHH activity (*23*). With respect to the C3H mouse strain, the activity for males and females is about 3-fold that of the male inbred SD rat. This places the C3H mouse in the approximate range of 350 to 450 activity units, with a small standard deviation (*24*).

Although it is instructive to note these comparisons, it is important not to lose sight of the total bioactivation/detoxification balance in various organs (e.g., liver) and the relationship to active carcinogens.

Hepatic/Skin Epoxide Hydrase Activity

Human. In a survey of 163 individuals (most with liver disease), there was a 63-fold range of hepatic epoxide hydrase (EH) activity (with benzo[a]pyrene 1,1-oxide as substrate). Approximately 80 to 90% of these people were within a 10-fold range of activity. The hepatic cytosol EH activity, determined with *trans*-stilbene oxide as substrate, varied over 539-fold among 135 subjects (*25*). No data were presented for the determination of what percentage of those studied fell within a 10-fold range of activity.

Animal. Hepatic microsomal levels of EH in humans have been compared with those of several commonly used animal models, including the mouse (NIH and CEH strain), rat (Sprague-Dawley), guinea pig (Hartley), rabbit (New Zealand), and rhesus monkey (*24,26*). In general, the mouse, regardless of strain, displays the lowest EH activity level. If the CEH mouse strain value is arbitrarily assigned a value of 1, then the values in the other species including humans become: rat–5.8, rabbit–6.2, guinea pig–13.5, monkey–31.5, and human–13.5.

The activity of EH in the healthy skin of one individual was measured for six substrates (*27*) and compared with rat and mouse values. In addition, the skin from six humans was assessed for its EH activity with respect to one of the above substrates (e.g., benzo[a]pyrene) to describe interindividual variation. In these individuals, variation in EH activity ranged from 175 to 447 μmole of product/min/mg protein. There was no obvious association with age, sex, or drug/medicinal factors; however, skin location may be a confounding factor, as the samples were obtained from the abdomen, thigh, and breast. In the interspecies comparison, the human always displayed a higher activity than the mouse, followed by the rat. The low value of 175 activity units was greater than the highest value of the rat or mouse. These data suggest that for this parameter, the human range of variation falls outside that of either rodent model.

Unscheduled DNA Synthesis Repair—Human Variation

Treatment of fibroblasts from presumably normal people (n = 10) with the carcinogens *N*-methyl-*N*-nitro-

N-nitrosoguanidine (MNNG) and *N*-acetoxy-2-acetylaminofluorene (NA-AAF) revealed a 2- to 6-fold interindividual variation in unscheduled DNA synthesis (UDS) (*28,29*). Homozygous individuals for xeroderma pigmentosum, a disease caused by impaired DNA repair, display only 10% of the DNA repair capability of normal persons when exposed to ultraviolet light, 4-nitroquinoline (4NQO), and AAF (*2*). Differences in the activities of both uracil DNA glycosylase and O^6-alkylguanine DNA transalkylase (i.e., DNA repair activities) are 3- to 65-fold in various human tissues (*22*). These findings, however, are based on a very modest sample of 12 persons. Harris et al. (*22*) speculated that "one can predict that studies measuring O^6-alkylguanine DNA transalkylase and other DNA repair enzymes in larger human populations will reveal even wider variations."

Carcinogen Binding to DNA

Human. TRACHEA/BRONCHUS. Benzo[a]pyrene (BP) binding to DNA in cultured human bronchial tissue was found to vary from 1- to 150-fold for $n = 19$ subjects (Fig. 4). The range of variation was about 9-fold for 80% of the group studied (*30*).

COLON. BP binding to DNA in cultured human colonic tissue has been found to vary over an approximately 30-fold range with $n = 63$. Approximately 90% of the individuals were within a 15-fold range (*31*). However, Autrup et al. (*32*) found a 100-fold variation in the binding of BP to human colonic tissue among 32 individuals. Approximately 80% of those studied ranged within a factor of 10 (Fig. 5).

ESOPHAGUS. BP binding to DNA in human esophageal tissue varied over a 20-fold range; however, only six subjects were studied (*22*).

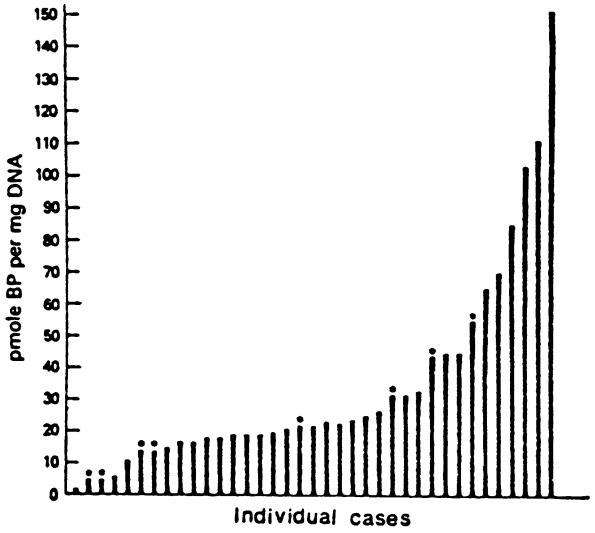

FIGURE 4. The interindividual variation in the binding levels of benzo[a]pyrene (BP) to DNA in cultured human bronchi. The values marked with an asterisk are from patients without lung cancer, and the remainder are from lung cancer patients (*30*).

FIGURE 5. Interindividual variation in the binding levels of benzo[a]pyrene (BP) to DNA in cultured human colon. The values marked with an asterisk are from colon explants prepared from immediate autopsies (*32*).

Animal. TRACHEA/BRONCHUS. While only limited direct animal model comparisons with humans have been conducted, Autrup et al. (*33*) found that the Syrian golden hamster displayed about 2.5 times as much binding of BP to tracheal DNA as did the human. The human variation ranged from 3 to 17 units, or a nearly 6-fold range of variation. Little variation occurred for the hamster (26–27 units). Mouse (DBA strain) and rat (CD strain) trachea activity averaged 9.25 with a narrow range of variation of 9 to 11 units. In contrast to the previous study, Daniel et al. (*34*) revealed similar binding capacity of BP to DNA in humans and hamsters with the rat, dog, and monkey showing progressively lower binding.

COLON. Autrup et al. (*35*) evaluated the capacity of three carcinogens [i.e., aflatoxin (AFB), benzo[a]pyrene (BP), and dimethylhydrazine (DMH)] to bind to the DNA of cultured rat (male CD strain, 4–5 weeks old) and human colonic tissue (i.e., nontumorous). They found that the mean level for binding of AFB and DMH to colonic DNA was significantly higher ($p < 0.01$) in the rat than in the human by 8.3-fold and 2-fold, respectively. No statistically significant differences were found for BP binding. Of great interest is the observation that the human colonic DNA displayed wide interindividual variation in binding levels for all three carcinogens [AFB (70-fold in 24 cases), BP (130-fold in 103 cases), and DNH (80-fold in 66 cases)]. In contrast, the variation seen in the animal tests was markedly smaller. For example, the standard deviations in the rat data with respect to a percentage of the mean value for AFB, BP, and DMH were 1.5, 8.2, and 2.3%, respectively. For humans the comparable values were 126, 99, and 103%.

β-Glucuronidase Activity

Tissue. The level of β-glucuronidase in multiple tissues has been studied in a variety of animal species as well as in humans. Table 1 provides a comparison of the β-glucuronidase activity of various mammalian tissues.

Table 1. Comparative β-glucuronide activity of mammalian tissues.[a]

Species	Intestinal contents: Small	Intestinal contents: Large	Liver	Kidney	Lung	Spleen	Brain	Large intestine	Small intestine	Stomach
Mouse High activity strains	—	—	2000–4000	1000–4500	—	4000–11,000	100	—	—	—
Mouse Low activity strains	—	—	200–400	250–900	—	1300	20	—	—	—
Rat	100–300	3000	15,000–30,000	4000–6000	5000	15,000–30,000	150	3500	3000–5000	—
Human	—	—	3000	2000	500	6500	Trace	—	—	200–10,000
Rabbit	10–250	2000	5000	300	—	—	150	300	800	40
Guinea pig	—	—	4500	600–1700	—	5500	300	—	—	—
Hamster	—	—	4500	1100	—	—	—	—	—	—

[a] Most common method for tissue bioassays. Measured as phenolphthalein liberated from phenolphthalein β-glucuronide in 1 hr at 38°C in microgram/gram moist tissue. Modified from Dutton (*36*).

Rat liver and lung activity levels are 10-fold greater than human levels. Also, there are both low and high activity mouse strains with the low mouse strain displaying about 1/10 the activity of humans (*36*).

The excretion and possible detoxification of carcinogens such as DMBA are enhanced by glucuronidation and can be markedly decreased when the activity of β-glucuronidase is chemically inhibited by D-glucaro-1-4-lactone. Factors such as this become a major consideration when determining an individual's susceptibility to these carcinogens. Treatment with the lactone reduced the level of β-glucuronidase in that tissue by approximately 45% and reduced the incidence of mammary tumors by 70% in rats (*37*). The authors speculated that "inhibition of β-glucuronidase activity increases the proportion of DMBA which is sequestered and excreted as the glucuronide and therefore is unavailable for activation to the proximal carcinogen."

It may be speculated that humans should be at a lower risk than rats since they display about 10-fold less β-glucuronidase activity. The data available for assessing the extent to which humans vary in tissue β-glucuronidase activity are very limited. Nevertheless, several studies have indicated that wide variation does exist (*38,39*), and there is speculation that these variations contribute to the occurrence of differential susceptibility of cancer in the population.

Gastrointestinal Tract. Gastrointestinal tract (GI) levels of β-glucuronidase activity are also highly variable in humans and may markedly affect the response to some carcinogenic agents. β-Glucuronidase levels in the small intestine are principally derived from gut microflora and are affected by diet. Since the diet varies, it is likely that the gut microflora also vary in the type and quantity of microorganisms and the level of β-glucuronidase activity. Persons receiving antibiotic treatment would have a depressed level of β-glucuronidase activity, depending upon the action of the drug on gut microflora (*40*).

Many carcinogenic agents such as BP and DMBA are conjugated with glucuronic acid and excreted via the bile. It is important to note that compounds may become hydrolyzed by β-glucuronidase while in the small intestine and enter into enterohepatic circulation, thereby increasing their residence time in the body. Individuals with low β-glucuronidase activity in the small intestine would be expected to have a reduced enterohepatic circulation of agents conjugated with glucuronic acid and secreted in the bile.

In terms of commonly employed animal models, it should be pointed out that mice and rats, respectively, are estimated to have approximately 60,000- and 15,000-fold greater β-glucuronidase activity in the proximal small intestine than humans (*41*). Such findings suggest that these models may display considerably greater enterohepatic circulation of carcinogens conjugated with glucuronic acid and excreted via the bile. As seen in Table 2, the rabbit and guinea pig models offer a closer approximation to the human even though these species far exceed estimated human values (*41*).

Biological Basis for Expanding Current Extrapolation Procedures

The previous examples indicate that there is extensive variation of the biochemical parameters believed to be associated with important phases in the process of carcinogenesis, including bioactivation and deactivation, in the human population. The extent of this variation differs with the specific parameter and is likely to be related to the sample size and characteristics of the population from which samples were derived. The human population from which samples have been derived is usually less than several hundred individuals and at times may be less than 20. In addition, the samples employed may not be representative of a larger population group. Investigators frequently have little

Table 2. Estimated β-glucuronidase activity in the small intestine of the human and four laboratory animal species.[a]

Species	Estimated β-glucuronidase activity, μg/g tissue: Proximal small intestine	Distal small intestine
Human	0.02	0.9
Rabbit	2.4	45.4
Guinea pig	2.7	139.0
Rat	304.0	1341.0
Mouse	1200.0	5015.0

[a] Modified from Draser and Hill (*41*).

choice but to conduct these studies on the basis of human specimens received by happenstance. Consequently, although the data indicate that human ability to bioactivate certain mutagens/carcinogens varies, it is not possible to make confident generalizations about either the extent of this heterogeneity in the human population or the distribution of the range of effect. Available data indicate that human variation in response to carcinogenic agents is generally much greater than that observed in commonly studied laboratory species. Most reports seldom state which segment of the human population the animal model is thought to represent, although it is generally assumed that the model represents the population mean.

The examples described in this paper expand the general approach by illustrating how information about a group of humans and various animal models can be extrapolated. For example, with respect to acetylation, the most appropriate model depends on the human phenotype. While it is necessary to combine this information with variations in the ability to *N*-hydroxylate and deacetylate arylamines to provide an accurate estimate of arylamine-induced cancer risk, this type of assessment affords a new population-based interpretational approach to determining the human significance of bioassay data.

The recognition that heterogeneity of response to the expression of biochemical parameters associated with the risk of developing chemically induced cancer in the human population presents the opportunity to use interspecies differences as an asset rather than a liability. For example, it could be that one animal model may reasonably simulate the response of one segment of the population (e.g., fast acetylator), while another model may best simulate another segment of the population (e.g., slow acetylator). Another approach may be for the testing agency to select the animal model on the basis of which segment of the population that it desires to simulate. Thus, it may be possible to select an animal model that more closely simulates a potential human high-risk group. This type of knowledge may be of considerable value for interpreting estimates of cancer risk to humans when these are based on biomathematical models.

The approach described in this paper may be of assistance in the development of prospective assessments, in the development of new protocols for testing, and in the reinterpretation of past studies to assess their extrapolative value. For example, the results of cancer bioassay studies in the dog have been used by the Occupational Safety and Health Administration (OSHA) to quantitatively assess the bladder cancer risk from exposure to the carcinogenic agent MOCA (*42*). Available information indicates that MOCA must be *N*-hydroxylated and not acetylated and that the dog lacks the ability to acetylate aromatic amines. The fact that the dog has a more favorable deacetylase/acetylase ratio than humans for the metabolism of aromatic amines would suggest that the dog is likely to overpredict human risk.

Summary

The relationship between human heterogeneity and susceptibility to the effects of toxic agents, including carcinogens, has become increasingly recognized. Identification of the causes of differential susceptibility is presently an active area of biomedical/toxicological research. The highly varied susceptibility of humans to environmental agents can be related to the fact that humans are a highly outbred species that follow a broad variety of dietary patterns and divergent lifestyles. In contrast, the responses of humans to toxic agents are currently predicted from highly inbred rodent strains raised on standardized diets and housed under similar environmental conditions. This paper describes an approach for addressing this principal issue of animal extrapolation with a perspective for biologically plausible interpretations of cancer bioassay data.

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REFERENCES

1. Calabrese, E. J. Pollutants and High Risk Groups: The Biological Basis of Enhanced Susceptibility to Environmental and Occupational Pollutants. John Wiley and Sons, New York, 1978.
2. Calabrese, E. J. Ecogenetics: Genetic Variation in Susceptibility to Environmental Agents. John Wiley and Sons, New York, 1984.
3. Calabrese, E. J. Toxic Susceptibilities: Male/Female Differences. John Wiley and Sons, New York, 1985.
4. Calabrese, E. J. Age and Susceptibility to Toxic Substances. John Wiley and Sons, New York, 1987.
5. Propping, P. Pharmacogenetics. Rev. Physiol. Biochem. Pharmacol. 83: 123–173 (1978).
6. National Research Council, Safe Drinking Water Committee. Drinking Water and Health. National Academy of Sciences, Washington, DC, 1977, pp. 803–804.
7. Dourson, M. L., and Stara, J. J. Regulatory history and experimental support of uncertainty (safety) factors. Regul. Toxicol. Pharmacol. 3: 224–238 (1983).
8. U.S. Environmental Protection Agency, Office of Water Supply. National Interim Primary Drinking Water Regulations. EPA 570/9-76-003. National Technical Information Service, U.S. Department of Commerce, Springfield, VA, 1976.
9. Glowinski, I. R., Radtke, H. E., and Weber, W. W. Genetic variation in N-acetylation of carcinogenic arylamines by human and rabbit liver. Mol. Pharmacol. 14: 940–949 (1978).
10. Lower, G. M., Jr., and Bryan, G. T. Enzymatic N-acetylation of carcinogenic aromatic amines by liver cytosol of species displaying different organ susceptibilities. Biochem. Pharmacol. 22: 1581–1588 (1973).
11. Devadatta, S., Gangadharam, P. R. J., Andrews, R. H., Fox, W., Ramakrishnan, C. V., Selkon, J. B., and Velu, S. Peripheral neuritis due to isoniazid. Bull. WHO 23: 587–598 (1960).
12. Mitchell, J. R., Zimmerman, H. J., Ishak, K. G., Thorgeirsson, U. P., Timbrell, J. A., Snodgrass, W. R., and Nelson, S. D. Isoniazid liver injury: Clinical spectrum, pathology, and probable pathogenesis. Ann. Intern. Med. 84: 181–192 (1976).
13. Lower, G. M., Jr., Nilsson, T., Nelson, C., Wolf, H., Gamsky, T. E., and Bryan, G. T. N-acetyltransferase phenotype and risk in urinary bladder cancer: Approaches in molecular epidemiology. Preliminary results in Sweden and Denmark. Environ. Health Perspect. 29: 71–79 (1979).
14. Lower, G. M., Jr., and Bryan, G. T. Enzymic deacetylation of carcinogenic arylacetamides by tissue microsomes of the dog and other species. J. Toxicol. Environ. Health 1: 421–432 (1976).
15. Williams, R. T. Detoxification Mechanisms: The Metabolism and

Detoxication of Drugs, Toxic Substances and Other Organic Compounds, 2nd ed. Chapman and Hall, London, 1959, p. 438.
16. Idle, J. R., and Smith, R. L. Polymorphisms of oxidation at carbon centers of drugs and their clinical significance. Drug Metab. Rev. 9: 301–317 (1979).
17. Evans, D. A. P., Mahgoub, A., Sloan, T. P., Idle, J. R., and Smith, R. L. A family and population study of the genetic polymorphism of debrisoquine oxidation in a white British population. J. Med. Genet. 17: 102–105 (1980).
18. Mahgoub, A., Idle, J. R., Dring, L. G., Lancaster, R., and Smith, R. L. Polymorphic hydroxylation of debrisoquine in man. Lancet ii: 584–586 (1977).
19. Mbanefo, C., Bababunmi, E. A., Mahgoub, A., Sloan, T. P., Idle, J. R., and Smith, R. L. A study of the debrisoquine hydroxylation polymorphism in a Nigerian population. Xenobiotica 10: 811–818 (1980).
20. Al-Dabbagh, S. G., Idle, J. R., and Smith, R. L. Animal modelling of human polymorphic drug oxidation: The metabolism of debrisoquine and phenacetin in rat inbred strains. J. Pharm. Pharmacol. 33: 161–164 (1981).
21. Kouri, R. E., Levine, A. S., Edwards, B. K., McLemore, T. L., Vesell, E. S., and Nebert, D. W. Source of interindividual variations in aryl hydrocarbon hydroxylase in mitogen-activated human lymphocytes. In: Genetic Variability in Response to Chemical Exposure, Banbury Report No. 16 (G. S. Omenn and H. V. Gelboin, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984, pp. 131–143.
22. Harris, C. C., Autrup, H., Vahakangas, K., and Trump, B. F. Interindividual variation in carcinogen activation and DNA repair. In: Genetic Variability in Responses to Chemical Exposure, Banbury Report No. 16 (G. S. Omenn and H. V. Gelboin, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984, pp. 145–153.
23. Pelkonen, O., Kaltiala, E. H., Karki, N. T., Jalonen, K., and Pyorala, K. Properties of benzpyrene hydroxylase from human liver and comparison with the rat, rabbit and guinea-pig enzymes. Xenobiotica 5: 501–509 (1975).
24. Oesch, F., Jerina, D. M., Daly, J. W., and Rice, J. M. Induction, activation and inhibition of epoxide hydrase: An anomalous prevention of chlorobenezene-induced hepatotoxicity by an inhibitor of epoxide hydrase. Chem.-Biol. Interact. 6: 189–202 (1973).
25. Glatt, H., and Oesch, F. Variations in epoxide hydrolase activities in human liver and blood. In: Genetic Variability in Response to Chemical Exposure, Banbury Report No. 16 (G. S. Omenn and H. V. Gelboin, Eds.), Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1984, pp. 189–203.
26. Oesch, F. Mammalian epoxide hydrases: Inducible enzymes catalysing the inactivation of carcinogenic and cytotoxic metabolites derived from aromatic and olefinic compounds. Xenobiotica 3: 305–340 (1972).
27. Oesch, F., Schmassmann, H., and Bentley, P. Specificity of human, rat and mouse skin epoxide hydratase towards K-region epoxides of polycyclic hydrocarbons. Biochem. Pharmacol. 27: 17–20 (1978).
28. Lake, R. S. Individual variation in DNA repair in human peripheral blood monocytes. In: Individual Susceptibility to Genotoxic Agents in the Human Population (F. J. De Serres and R. W. Pero, Eds.), Plenum Press, New York, 1984, pp. 349–358.
29. Pero, R. W. Unscheduled DNA synthesis induced by N-acetoxy-2-acetylamino fluorine as an indicator of risk from genotoxic exposures. In: Individual Susceptibility to Genotoxic Agents in the Human Population (F. J. De Serres and R. W. Pero, Eds.), Plenum Press, New York, 1984, pp. 333–347.
30. Harris, C. C., Autrup, H., Connor, R., Barrett, L. A., McDowell, E. M., and Trump, B. F. Interindividual variation in binding of benzo[a]pyrene to DNA in cultured human bronchi. Science 194: 1067–1069 (1976).
31. Harris, C. C., Mulvihill, J. J., Thorgeirsson, S. S., and Minna, J. D. Individual differences in cancer susceptibility. Ann. Intern. Med. 92: 809–825 (1980).
32. Autrup, H., Barrett, L. A., Jackson, F. E., Jesudason, M. L., Stoner, G., Phelps, P., Trump, B. F., and Harris, C. C. Explant culture of human colon. Gastroenterology 74: 1248–1257 (1978).
33. Autrup, H., Wefald, F. C., Jeffrey, A. M., Tate, H., Schwartz, R. D., Trump, B. F., and Harris, C. C. Metabolism of benzo[a] pyrene by cultured tracheobronchial tissues from mice, rats, hamsters, bovines and humans. Int. J. Cancer 25: 293–300 (1980).
34. Daniel, F. B., Schut, H. A. J., Sandwisch, D. W., Schenck, K. M., Hoffmann, C. O., Patrick, J. R., and Stoner, G. D. Interspecies comparisons of benzo[a]pyrene metabolism and DNA-adduct formation in cultured human and animal bladder and tracheobronchial tissues. Cancer Res. 43: 4723–4729 (1983).
35. Autrup, H., Schwartz, R. D., Essigmann, J. M., Smith, L., Trump, B. F., and Harris, C. C. Metabolism of aflatoxin B_1, benzo[a]pyrene, and 1,2-dimethylhydrazine by cultured rat and human colon. Teratog. Carcinog. Mutagen. 1: 3–13 (1980).
36. Dutton, G. J., Ed. Glucuronic Acid: Free and Combined. Academic Press, New York, 1966.
37. Walaszek, Z., Hanausek-Walaszek, M., and Webb, T. E. Inhibition of 7,12-dimethylbenzanthracene-induced rat mammary tumorigenesis by 2,5-di-O-acetyl-D-glucaro-1,4: 6,3-dilactone, an *in vivo* β-glucuronidase inhibitor. Carcinogenesis 5: 767–772 (1984).
38. Lomdardo, A., Goi, G. C., Marchesini, S. Caima, L., Moro, M., and Tettamanti, G. Influence of age and sex on five huma plasma lysosomal enzymes assayed by automated procedures. Clin. Chim. Acta 113: 141–152 (1981).
39. Maire, I., Mandon, G., Zabot, M. T., Mathieu, M., and Guibaud, P. β-Glucuronidase deficiency: Enzyme studies in an affected family and prenatal diagnosis. J. Inherited Metab. Dis. 2: 29–34 (1979).
40. Calabrese, E. J. Principles of Animal Extrapolation. John Wiley and Sons, New York, 1983.
41. Drasar, B. S., and Hill, M. J. Bacterial glycosidases. In: Human Intestinal Flora. Academic Press, New York, 1974, pp. 54–71.
42. Stula, E. F., Barnes, J. R., Sherman, H., Reinhardt, C. F., and Zapp, J. A., Jr. Urinary bladder tumors in dogs from 4,4′-methylene-bis(2-chloroaniline) (MOCA). J. Environ. Pathol. Toxicol. 1: 31–50 (1977).